

Instructions for CellProfiler™ Cell Image Analysis Software* to Analyze Data from Radius™ Cell Migration Assays ***Calcein AM***

The following instructions and figures are provided as a guide to aid in Radius™ Cell Migration Assay data quantitation. They are designed to analyze single, 10X magnification images from the Radius™ Cell Migration Assay Kit that have been stained Calcein AM. For each image to be analyzed the image file name must be entered, and the manual threshold value must be optimized, which may require some trial and error. Please refer to the CellProfiler™ manual for more detailed instructions in its use and customization at <http://www.cellprofiler.org>. Please contact Cell Biolabs with any questions at tech@cellbiolabs.com.

1. Create and name a new folder (e.g. Radius™ Calcein AM) and copy over the files you downloaded along with these instructions (*3 Sample Calcein AM image files and the Radius Assay Calcein AM.cp file*).
2. Download and install the CellProfiler™ Cell Image Analysis Software:
<http://www.cellprofiler.org/download.shtml>.
3. Launch the CellProfiler™ software.
4. Set both the Default Input and Output Folders (lower right corner of the main window) to the folder containing the images you want to analyze (for sample images refer to folder created in step 1).
5. Check the Allow overwrite box (lower right corner of the main window).
6. Load the custom pipeline file by doing one of the following:
 - Double-click on the *Radius Assay Calcein AM.cp* file in the lower left window, then click “Yes”.
 - Click *File > Load Pipeline*. Browse to the file *Radius Assay Calcein AM.cp* in the folder created in step 1, then click “Open”.
7. Select the LoadImages Module (upper left)
 - In the right settings window, enter the image name into the field “*Text that these images have in common*”. Available images can be found in the lower left window. Enter the file name to be analyzed exactly as it appears in the window, including extension (e.g. Sample Calcein AM 1.jpg, Sample Calcein AM 2.jpg, Sample Calcein AM 3.jpg). You will need to type it, as the CellProfiler program does not have a browse feature for this step.
Note: This step will need to be done for each image to be analyzed.
8. Select the IdentifyPrimaryObjects Module (upper left)
 - In the right settings window, enter the threshold value into the “*Manual threshold*” field.
Note: This will need to be optimized for each image. Values may range from 0-1 and require some manipulation to determine the best fit. For the Sample Calcein AM Images 1, 2 and 3 we provided, use a manual threshold of 0.80, 0.55 and 0.65 respectively.
9. Select the Analyze images button from the bottom right. An analysis summary window appears. This analysis summary may be saved as an image file by clicking *File > Save > Save as Png Image*.

*CellProfiler™ cell image analysis software is a free program owned and offered by the Broad Institute. There is no relationship between Cell Biolabs, Inc and the Broad Institute. Cell Biolabs offers these instructions as a courtesy to our customers who wish to analyze data obtained using our Radius™ Cell Migration Assays.

Examples of Results

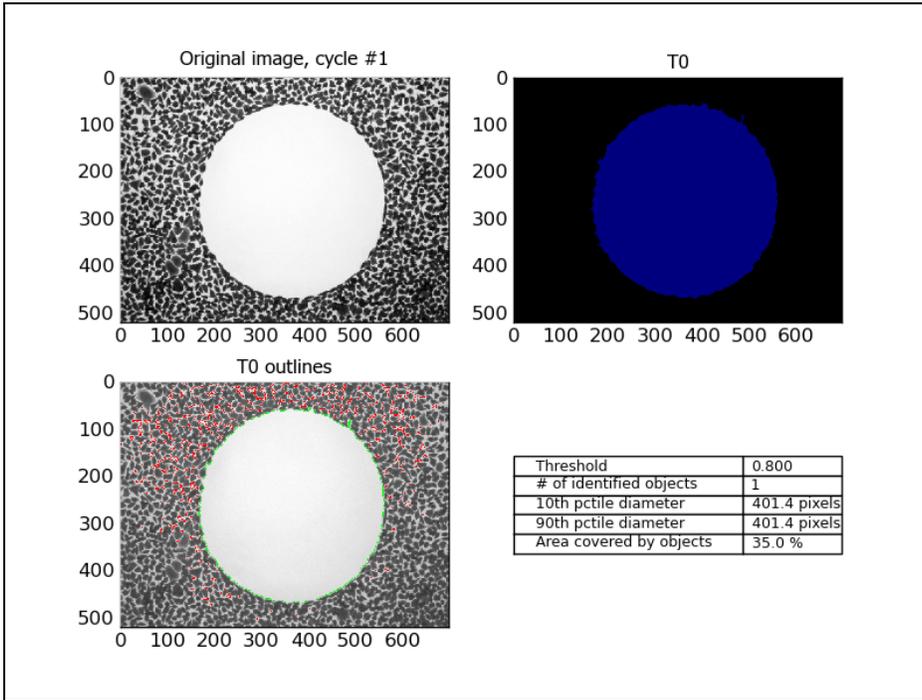


Figure 1: Sample Cell Stain 1.jpg analyzed with manual threshold value of 0.80.

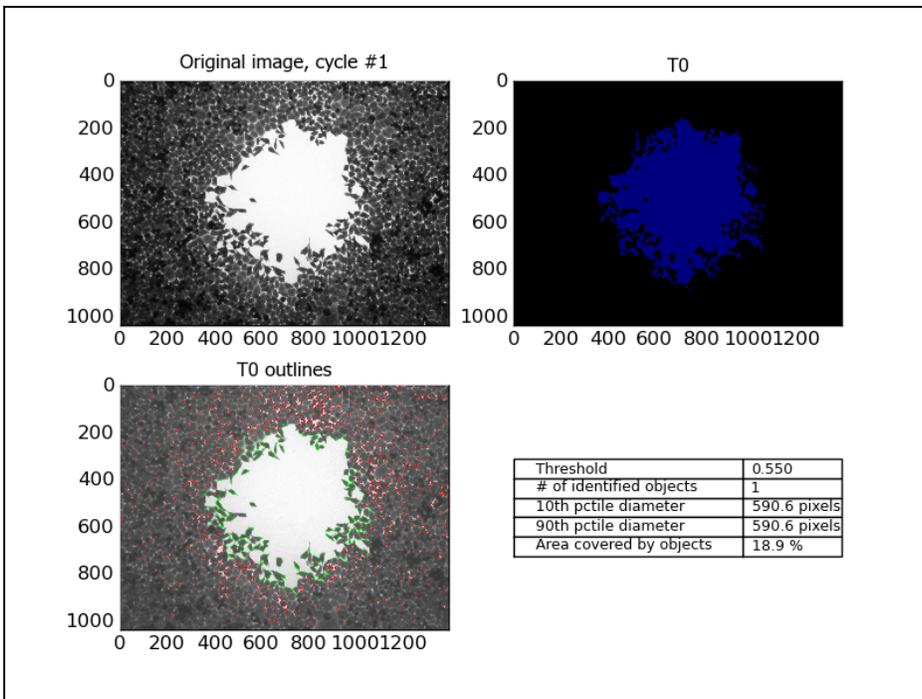


Figure 2: Sample Cell Stain 2.jpg analyzed with manual threshold value of 0.55.

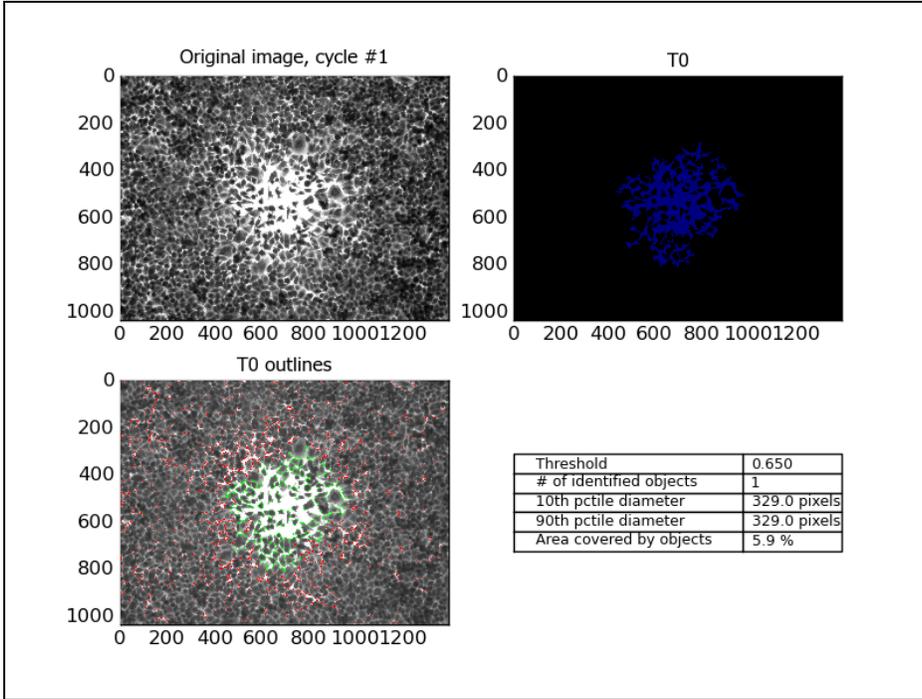


Figure 3: Sample Cell Stain 3.jpg analyzed with manual threshold value of 0.65.